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128850

MRID No. 413961-09

DATA EVALUATION RECORD

1. **CHEMICAL:** Glufosinate.
Shaughnessey No. 128850.

2. **TEST MATERIAL:** HOE 039866 200 g/L Soluble Concentrate; Code #HOE 039866 OH SL18 A507; 18.5% active ingredient; a white powder.

3. **STUDY TYPE:** Mollusc 48-hour Embryo-Larval Study.
Species Tested: Eastern Oyster (Crassostrea virginica).

4. **CITATION:** Ward, G.S. 1989. Acute Toxicity of HOE 039866 200 g/L Soluble Concentrate (Code: HOE 039866 OH SL18 A507) to Embryos and Larvae of the Eastern Oyster (Crassostrea virginica). Prepared by Hunter/ESE, Gainesville, Florida. ESE Project No. 87341-0210-2130. Submitted by Hoechst Celanese Corporation, Somerville, New Jersey. MRID No. 413961-09.

5. **REVIEWED BY:**

Kimberly D. Rhodes
Associate Scientist
KBN Engineering and
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Signature: Kimberly D. Rhodes
Date: June 1, 1990

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
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Signature: P. Kosalwat
Date: 6/1/90

Henry T. Craven, M.S.
Supervisor, EEB/HED
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Signature: A C. Craven M. Rhodes
Date: 12/20/90 12/24/90

7. **CONCLUSIONS:** This study appears scientifically sound but does not fulfill the guideline requirements for an oyster embryo-larval test. Only one test concentration had an inhibitary effect on the test animals. Therefore, an EC50 could not be determined from this study. The NOEC was determined to be 1.62 mg/L, based on whole material, after 48 hours of exposure.

No EC50

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Adult Eastern oysters (Crassostrea virginica) were obtained from the Horn Point Environmental Laboratory of the University of Maryland. The oysters were maintained in natural seawater at a salinity of 20 parts per thousand (ppt) and a temperature of 20 to 22°C for 3 days prior to spawning.

Individual, sexually mature female oysters were induced to spawn by placing them in 1.6-liter (L) glass chambers containing 1 L of dilution water at approximately 23°C and increasing the water temperature to approximately 30°C in the presence of viable sperm stripped and/or released from a sexually mature male oyster. Fertilization occurred upon release of the eggs into the spawning chambers and was confirmed microscopically.

B. **Test System:** The test was performed in 1-L glass beakers containing 0.9 L of test solution. All test concentrations and the controls were triplicated. The test containers were maintained at 22 ± 1°C under fluorescent lighting on a photoperiod of 16 hours of light and 8 hours of darkness.

The dilution water was filtered natural seawater collected from the Atlantic Ocean near Marineland, Florida, and diluted to a salinity of approximately 19 ppt with well water. The water was filtered through a 0.45 µm filter membrane prior to addition to the test containers. The dilution water control was characterized as having a dissolved oxygen concentration of 7.4, a pH of 7.9, and a salinity of 19 ppt.

C. **Dosage:** Mollusc 48-hour embryo-larval static test.

D. **Design:** Based on the results of a range-finding test, a control and six nominal HOE 039866 200 g/L soluble concentrate concentrations of 0.21, 0.35, 0.58, 0.97, 1.62, and 2.70 mg/L were chosen for testing. The nominal concentrations were based on whole material. Each test container was inoculated with an estimated

27,000 embryos within 1 hour of fertilization. Initial embryo density was determined by Sedgewick-Rafter counts of three 10-mL subsamples removed from the triplicate control flasks. Counts of embryos added to test containers at test initiation indicated the initial inoculum was actually only 20,370 on the average.

After 48 hours of exposure, 10-mL samples were collected by automatic pipet while agitating with a perforated plunger. The samples containing the larvae were preserved with 0.4 mL of buffered formalin. The number of normally developed 48-hour larvae was determined by a Sedgewick-Rafter counter from each triplicate test and control container.

The dissolved oxygen concentration and pH were measured and recorded at 0 and 48 hours in all replicates for all test concentrations and the control. The salinity was measured in one seawater control test container at test initiation. The temperature was measured and recorded hourly by a computerized temperature data logger.

E. **Statistics:** Results of the toxicity test were used to calculate the percentage reduction of normal oyster larvae from each test concentration when compared to the control. The percentage reduction of normal 48-hour embryos was determined as follows:

$$\% \text{ Reduction} = \frac{\text{Mean number of normal larvae in each test concentration} - \text{Mean number of normal control larvae}}{\text{Mean number of normal control larvae}} \times 100$$

The numbers of normally developed larvae in the control were compared to the numbers of normally developed larvae in the test substance treatments to determine if exposure to any test concentration reduced the number of embryos developing normally. One-way analysis of variance (ANOVA) was conducted to determine if there was a significant difference among treatments. Dunnett's multiple comparison test was used to identify those test concentrations producing effects different from the control at a confidence level of 95 percent.

Based on the results of the test with HOE 039866 200 g/L soluble concentrate, the 48-hour EC50 value was estimated by graphical interpolation.

12. **REPORTED RESULTS:** Table 3-1 (attached) shows the number of normally developed larvae after 48 hours of exposure to HOE 039866 200 g/L soluble concentrate Technical and percentage reduction as compared to the control. HOE 039866 200 g/L soluble concentrate was acutely toxic to embryos and larvae at a nominal test concentration of 2.70 mg/L as whole product (0.49 mg/L as active ingredient). The percentage reduction of normal larvae, as compared to the control, after 48 hours of exposure was 52 percent in 2.70 mg/L. An increase in the number of normal larvae (ranging from 9 to 33%) occurred in all concentrations $\leq$1.62 mg/L nominal concentration. The 48-hour EC50 was 2.7 mg/L nominal concentration. The no-observed-effect concentration (NOEC) was 1.62 mg/L nominal concentration.

Test salinity was 19 ppt. The mean temperature was 22°C with a standard deviation of 1°C; temperature ranged from 20 to 24°C. The dissolved oxygen concentrations remained $\geq$6.8 mg/L ($\geq$89% of saturation) in all test solutions throughout the test. The pH of all test solutions remained between 7.8 and 8.1 during the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o The SEP states that natural or reconstituted seawater of 10 to 17 ppt salinity should be used when testing estuarine (euryhaline) mollusc species. The salinity of the seawater used in this toxicity test was 19 ppt.

o The SEP recommends a temperature of 20°C and states that the temperature should not vary more than 2°C during the test. During this toxicity test, the temperature ranged from 20 to 24°C.

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether 15- to 30-minute transition periods between light and dark were maintained.

o There is a discrepancy in the report involving the percent saturation of the dissolved oxygen concentration. The author determined the percent saturation for the lowest dissolved oxygen concentration (6.8 mg/L) to be 89 percent. However, the reviewer determined the percent saturation for the lowest dissolved oxygen concentration to be 79 percent at 19 ppt and 22°C.

B. **Statistical Analysis:** The reviewer could not perform statistical analysis since only the highest concentration (2.70 mg/L) showed a reduction of normal oyster embryo-larvae (52%). Higher test concentrations should have been conducted in this toxicity test to result in inhibitory effects in more than one test concentration. Thus an EC50 value could be determined.

C. **Discussion/Results:** Although the range finding test concentrations of 0.54, 5.4, and 54 mg/L resulted in reductions of normal larvae of 44%, 73%, and 96%, respectively, the concentrations selected for the definitive test were between 0.21 and 2.7 mg/L. The author did not provide any rationale why such a low range of concentrations were tested. Only the highest test concentration of 2.70 mg/L had a detrimental effect on the test animals. Therefore, an EC50 could not be determined from this study. The NOEC was determined to be 1.62 mg/L based on whole material.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental

(2) **Rationale:** Only the highest test concentration of 2.7 mg/L had a detrimental effect on the test animals. Therefore, an EC50 could not be determined from this study.

(3) **Repairability:** None.

15. **COMPLETION OF ONE-LINER:** Yes, 05-17-90.

Shaughnessey No. 28850 Chemical Name Glufosinate (HOE 039866 200g/L Soluble Concentrate) Chemical Class ____ Page ____ of ____

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Valid Sta
14-Day Single Dose Oral LD_{50}		LD50 = . mg/kg (95% C.L.) Contr. Mort.(%)=		
Species ____		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
Species ____		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
48-Hour EC_{50}		EC50 = * ppm (95% C.L. —) Contr. Reduction Mort.(%)= N/A Sol. Contr. Mort.(%)= N/A		
Species Crassostrea virginica 18.5‰		Slope= N/A # Animals/Level= 20, 370/mL Temperature (20-24°C)		
Lab Hunter/ESE		48-Hour Dose Level ppm /(%~~Mortality~~) Reduction 0.2 433 | 0.35 (+25) | 0.58 (+9) | 0.97 (+22) | 1.62 (+19) 2.7 (-52)		
Acc. 413961-09		Comments: * An EC50 could not be determined since only one concentration showed inhibitory effect.		
96-Hour LC_{50}		LC50 = ppm (95% C.L.) Con. Mort.(%)= Sol. Con. Mor.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab ____		96-Hour Dose Level ppm /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
96-Hour LC_{50}		LC50 = ppm (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab ____		96-Hour Dose Level ppm /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		

RCG 12/11/90 K.R. Supplemental 5/17/90

Table 3-1. Number of Normally Developed Larvae After 48 Hours of Exposure to HOE 039866 200 g/L Soluble Concentrate and Percentage Reduction as Compared to the Control

Nominal Concentrations (mg/L; ppm as whole product	Mean Number of Normal Larvae		Percentage Reduction of Normal 48-Hour Larvae (%)
	Mean	SD*	
Control	16,650	1,838	---
0.21	22,140	3,129	+33**
0.35	20,790	1,429	+25
0.58	18,210	699	+9
0.97	20,340	2,746	+22
1.62	21,660	2,259	+19
2.70	7,980	3,258	-52**

*SD = Standard deviation.
**Statistically different ($P \geq 0.95$) than the control.

Source: ESE, 1987.